A Reliable and Sustainable Multicomponent Access to Protein Degraders

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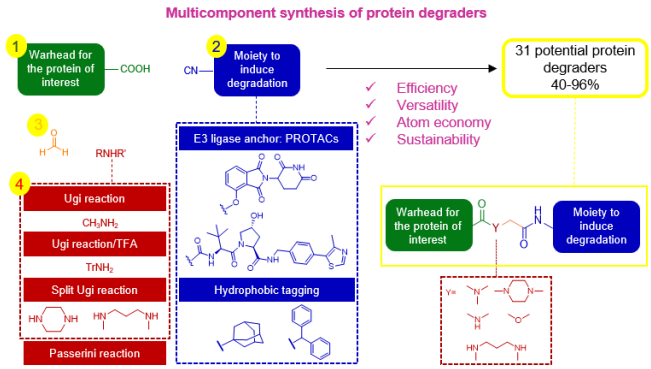
Graphical abstract



Abstract

The use of small molecules to induce targeted protein degradation is increasingly growing in the drug discovery landscape and protein degraders have progressed rapidly through the pipelines. Despite the advances made so far, their synthesis still represents a significant burden and new approaches are highly demanded. Herein, we report an unprecedented platform that leverages the modular nature of both multicomponent reactions and degraders to enable the preparation of highly decorated

PROTACs and hydrophobic tag-mediated degraders. Compared to the existing methods, our approach offers a versatile and cost-effective means to access libraries of protein degraders and increase the chance of identifying successful clinical candidates.

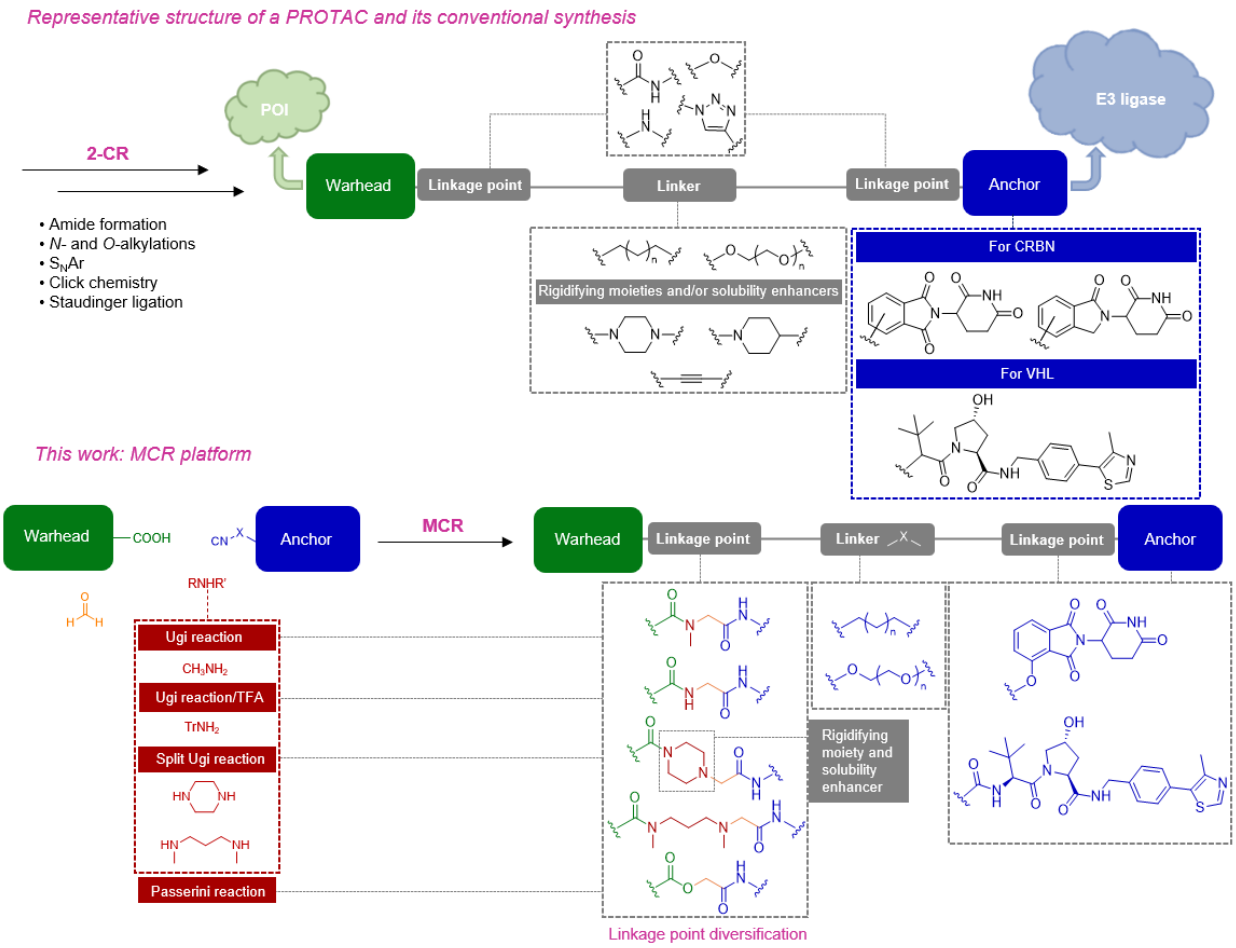
Keywords

PROTACs; protein degraders; hydrophobic tagging; multicomponent reactions; Ugi reaction; Passerini reaction.

Protein degraders represent a new strategy to reduce expression of disease-related proteins and are set to revolutionize drug discovery[1,2]. Among them, PROTACs (PRoteolysis TArgeting Chimeras) have drawn attention as one of the most appealing approaches[3]. Being composed of a ligand for the protein of interest (POI) (referred to as warhead), a linker and a ligand for the E3 ubiquitin ligase (referred to as anchor), PROTACs are able to hijack the ubiquitin-proteasome cascade and force the target protein to be degraded (Figure 1). Upon completion of the cycle, they are still available for successive rounds of degradation, thus minimizing the need for a continuous drug administration[4]. Over one hundred different proteins have been targeted so far, and even those proteins traditionally considered "undruggable" have been shown to be degraded by this system[5]. Noteworthy, compared to occupancy-driven approaches, PROTACs allow to abrogate all the activities associated with the selected protein. Their potential to knock down disease-related proteins is exemplified by the first degraders that have reached human clinical trials[6–8].

From a structural point of view, the warhead is represented by a ligand that binds the POI, independently from its mechanism of action. Concerning the anchor for the E3 ligases, cereblon (CRBN) targeting ligands (*i.e.* IMiD-based ligands, such as thalidomide and its structural analogues)[9] and Von Hippel-Lindau (VHL) targeting ligands (*i.e.* hydroxyproline-based molecules)[4] are the most commonly used binders. Given that there is relatively limited room of maneuver to alter the warhead and the anchor, synthetic modifications are focused on both the linker, that can be of different length and composition (*i.e.* linear aliphatic linkers, PEG and extended glycol chains), and the linkage points, whose structure is usually driven by the chemical reactions used to assemble the PROTAC rather than by a rational choice (*i.e.* amines, ethers, amide moieties). The sites of attachment of the linker to the anchor and the warhead are critical and, if possible, are selected by analyzing the solvent-exposed regions, in order to minimize the interference of the linker with the binding of the PROTAC to the E3 ligase and the POI, respectively (Figure 1). Only when all these structural requirements are found in the optimal combination, they engage in the productive formation of the ternary E3 ligase-PROTAC-POI complex and activate the proteasome cascade[10,11].

Fig. 1 – Representative structure of a PROTAC, its two-component reaction (2-CR)-based synthesis and our multicomponent reaction (MCR)-based platform.



The structural complexity of the ternary complex (TC) makes it a formidable challenge to predict *a priori* which combination of the different synthons is the best one to achieve degradation of the POI, even if X-ray crystal structures, molecular modelling and dynamics might at times be of help[12–14]. Besides degradation efficiency, similarly to any other medicinal chemistry campaign, properties such as solubility, metabolic stability and cell permeability represent an additional challenge to achieve an optimal *in vivo* effect, with far more reason since PROTACs lie outside the rule-of-five space[15–17]. Hence, efforts have been devoted to rationally explore the role of the different structural elements in conferring favorable pharmacokinetic properties and a special attention has been given to the chemistry of the linker, whose optimization has a crucial impact on the ADME profile throughout the R&D process[15,18,19]. Linear aliphatic and PEG-based linkers are commonly used in the early phases

of development to assess the most favorable length to form an efficient ternary complex, while at a later stage they are usually refined and enriched with rigidifying motifs (*e.g.* piperazines, piperidines) to further improve pharmacokinetic parameters (Figure 1).

Despite the advances made so far in the rational design of PROTACs, the conversion of a ligand for the POI into an effective degrader *in vivo* remains rather empirical and based on a "trial and error" approach[20]. Extensive investigation of multiple warheads, anchors, linker types and lengths, and linkage points is needed since the early stage of PROTAC development. Nevertheless, the lack of reliable and economically sustainable synthetic methods represents an important caveat that necessarily limits the number of accessible compounds. The assemble of PROTACs has proven to be far from straightforward as it involves the asymmetric diversification of the two sides of the linker and their chemoselective reactions with the two protein binding motifs. This challenge is usually achieved through orthogonal conditions or protection/deprotection sequences. Linking strategies to couple the linker with the warhead and the anchor include amide bond formation, *N*- and *O*-alkylations, nucleophilic aromatic substitution, acylation and Williamson ether synthesis, transformations that are known to suffer from poor reactivity, low chemoselectivity and lack of atom economy (Figure 1)[21]. It results that the preparation of PROTACs often requires low-yielding and cumbersome multistep synthetic routes, makes extensive use of protecting groups, and relies on highly functionalized and costly building blocks.

To overcome these limitations, notable advances aimed at simplifying the access to protein degraders have been reported[19] and include the use of orthogonally protected bifunctional linkers[22], solid phase synthesis[23], click chemistry[24], Staudinger ligation[25] and others[26]. Nevertheless, none of these strategies has established itself as the ideal method so far, due to existing limitations. For instance, among the recent methods reported to ease the preparation of PROTACs, the click chemistry platform allows the preparation of both CRBN and VHL recruiting degraders, but the introduction of the triazole ring is well-known for its reluctance to scale-up[27]. Despite the advances made so far, the

synthesis of PROTACs still represents a significant synthetic burden, limiting the chemical space that can be accessed.

In an effort to streamline the discovery of successful PROTACs, we report a modular synthetic platform that capitalizes on the versatility of multicomponent reactions (MCRs) to assemble heterobifunctional protein degraders (Figure 1).

Results

We initially focused on the Ugi reaction[28,29], that stands out for its efficiency, versatility, high atom economy, and simple and environmentally friendly experimental procedures, where the only by-product is one water molecule per molecule of product.

Selection of synthons for the Ugi reaction.

For an initial proof of concept, we decided to focus on the following synthons as a model system: five different isocyanides bearing the thalidomide-based CRBN recruiting anchor (**12**-**16**, Figure 2), a carboxylic acid (**17**) bearing the warhead based on (+)-JQ1 (a well-known BET inhibitor), formaldehyde (**18**) as the carbonyl compound to minimize the interference of the linker with the binding to the POI and three different commercially available amines (methylamine **19**, tritylamine as surrogate of ammonia **25**, and piperazine **31**) (Figure 3). All these building blocks are commercially available, except for the isocyanides, synthesized following an in-house developed synthetic route.

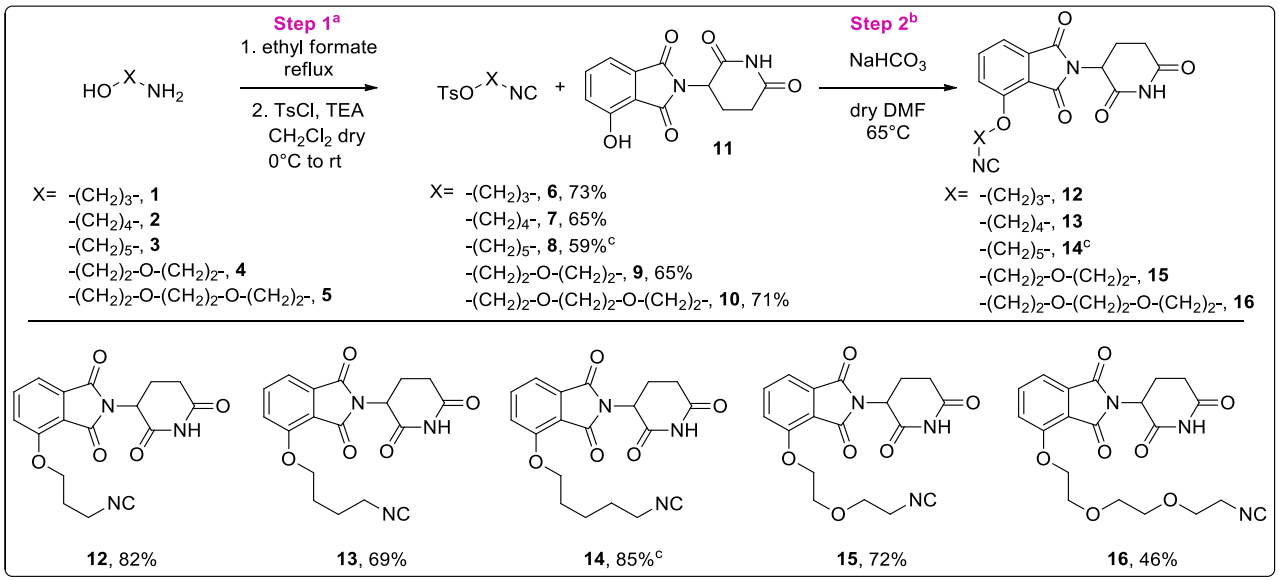
Preparation of the isocyanide-based CRBN recruiting anchors.

In the first step, an amino alcohol (**1-5**, Figure 2) is treated with ethyl formate and, upon completion of the reaction and after evaporation of the formylating agent, the resulting formamide is treated with tosyl chloride in the presence of triethylamine to give the heterobifunctional linker (**6-10**) bearing both the isocyanide and the tosylate in 59-73% yield. According to a recent report by Meier *et al.* published on *Green Chemistry*, tosyl chloride represents an efficient and practical alternative to toxic and hazardous dehydrating agents such as phosphorous oxychloride in the preparation of aliphatic isocyanides[30]. This method is perfectly suited to our purpose as it can simultaneously dehydrate the

formamide to isocyanide and tosylate the hydroxyl group. The third reaction is an *O*-alkylation that takes place by adding derivatives **6-10** to the thalidomide bearing a hydroxyl group at 4-position (**11**) in the presence of sodium bicarbonate. Notably, undesired substitution to the glutarimide nitrogen, that would result in the loss of E3 ligase recruitment, does not occur under our optimized conditions. By this strategy, both linear aliphatic (**12-14**) and PEG (**15-16**) linkers of different length have been prepared in yields spanning from 46% to 85%. The proposed reaction sequence stands out for its sustainability: all the required reagents are cheap and offer a simplified protocol to access the required linkers. Furthermore, as the isocyanides are stable upon long-term storage[31], they can be prepared and stocked to produce "user friendly" tool kits for the synthesis and diversification of PROTACs. Noteworthy, they do not suffer from the unpleasant smell typical of the isocyanides, due to the high molecular weight and solid state at room temperature.

With these linkers in our hands, we turned our efforts into the Ugi reaction, and we started investigating its use in PROTACs synthesis by varying the amine component.

Fig. 2 – Synthesis of the isocyanide-based library of CRBN recruiting anchors.



[a] Reactions were conducted on a 7.50 mmol scale with respect to **1-5**. Yields over two steps are given. Conditions: 1. Reactions were carried out with ethyl formate (5.00 mL) at reflux for 6 h. 2. Reactions were carried out with TsCl (22.5 mmol, 3 equiv), TEA (45.0 mmol, 6 equiv), and dry CH_2Cl_2 (14.0 mL) at room temperature for 5 h. [b] Reactions were

conducted on a 2.20 mmol scale with respect to **11**. Conditions: Reactions were carried out with $NaHCO_3$ (3.30 mmol, 1.5 equiv), isocyanide **6-10** (2.64 mmol, 1.2 equiv) and dry DMF (6.00 mL) at 65 °C overnight. [c] This reaction was scaled as described in Methods.

Different amines to probe the warhead linkage point.

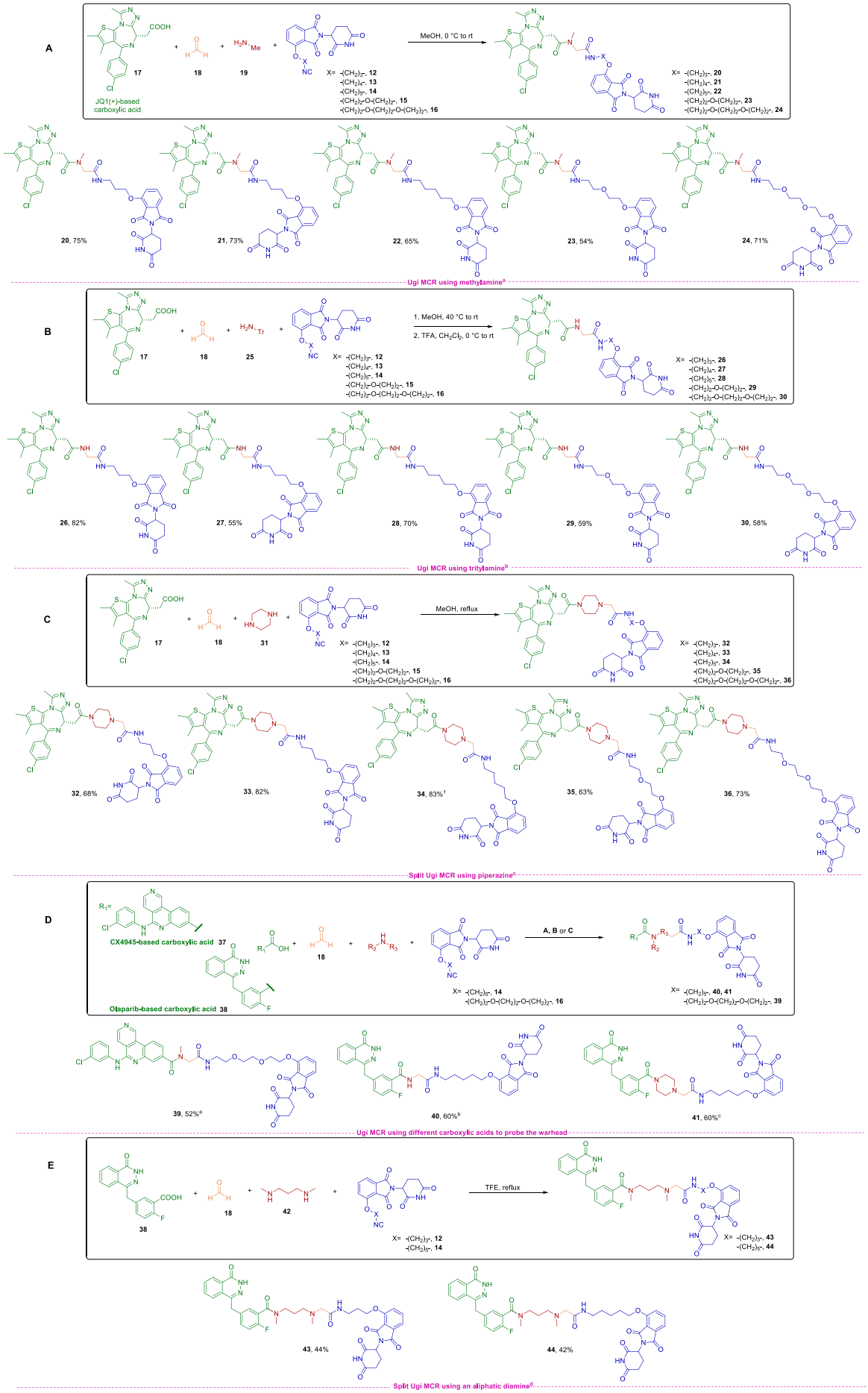
We began by methylamine (**19**) as a model for conventional primary amines and PROTACs **20**-**24** (Figure 3A) were synthesized in 54-75% yields.

In order to further reduce steric hindrance at the attachment point and minimally influence affinity of the warhead for its target protein, we then turned to explore the use of tritylamine (**25**), that our laboratory had recently reported as an effective surrogate of ammonia[32], especially when coupled with formaldehyde as the carbonyl component. The reaction was performed in methanol and, upon completion, the trityl group was cleaved by adding trifluoroacetic acid to give the desired PROTACs **26**-**30** in 55-82% yields (Figure 3B).

Finally, we exploited the split Ugi reaction that we reported in 2006[33] and is performed using a secondary amine, instead of a primary amine: one *N*-atom is acylated, while the other one is alkylated in one step, without the need for coupling agents or protecting groups. This variant particularly suites our purposes as it allows the one-pot introduction of the piperazine ring, a privileged substructure in PROTACs as a solubility enhancer, as a means to improve metabolic stability[15] and as a rigid moiety that leads to a stable ternary complex formation[34–36]. Noteworthy, the use of piperazine as the amine component allows for the increase of sp^3 character and the reduction of the number of HBDs and HBAs that have been associated with improved oral drug delivery. This is exemplified by the recently disclosed structures of ARV-110 for metastatic castration-resistant prostate cancer and ARV-471 for metastatic breast cancer that are two orally available PROTACs currently in clinical trials (NCT0888612 and NCT04072952)[37]. By means of the split Ugi reaction, PROTACs **32**-**36** were synthesized, with yields of 63-83% (Figure 3C).

To validate the scalability of the synthetic approach, the preparation of the isocyanide **14** was performed on a 6.00 mmol scale and the split Ugi reaction on a 1.50 mmol scale to afford 1 gram of **34** in 83% yield.

Fig. 3 – Library of PROTACs synthesized by varying the amine group and the carboxylic acid in the Ugi MCR.


A
JQ1(+)-based carboxylic acid
17
18
19
MeOH, 0 °C to rt
X= -(CH2)3-, 12
-(CH2)4-, 13
-(CH2)5-, 14
-(CH2)2-O-(CH2)2-, 15
-(CH2)2-O-(CH2)2-O-(CH2)2-, 16
X= -(CH2)3-, 20
-(CH2)4-, 21
-(CH2)5-, 22
-(CH2)2-O-(CH2)2-, 23
-(CH2)2-O-(CH2)2-O-(CH2)2-, 24
20, 75%
21, 73%
22, 65%
23, 54%
24, 71%
Ugi MCR using methylamine[a]
B
17
18
25
1. MeOH, 40 °C to rt
2. TFA, CH2Cl2, 0 °C to rt
X= -(CH2)3-, 12
-(CH2)4-, 13
-(CH2)5-, 14
-(CH2)2-O-(CH2)2-, 15
-(CH2)2-O-(CH2)2-O-(CH2)2-, 16
X= -(CH2)3-, 26
-(CH2)4-, 27
-(CH2)5-, 28
-(CH2)2-O-(CH2)2-, 29
-(CH2)2-O-(CH2)2-O-(CH2)2-, 30
26, 82%
27, 55%
28, 70%
29, 59%
30, 58%
Ugi MCR using tritylamine[b]
C
17
18
31
MeOH, reflux
X= -(CH2)3-, 12
-(CH2)4-, 13
-(CH2)5-, 14
-(CH2)2-O-(CH2)2-, 15
-(CH2)2-O-(CH2)2-O-(CH2)2-, 16
X= -(CH2)3-, 32
-(CH2)4-, 33
-(CH2)5-, 34
-(CH2)2-O-(CH2)2-, 35
-(CH2)2-O-(CH2)2-O-(CH2)2-, 36
32, 68%
33, 82%
34, 83%[1]
35, 63%
36, 73%
Split Ugi MCR using piperazine[c]
D
R1=
CX4945-based carboxylic acid 37
Olaparib-based carboxylic acid 38
18
A, B or C
X= -(CH2)5-, 14
-(CH2)2-O-(CH2)2-O-(CH2)2-, 16
X= -(CH2)5-, 40, 41
-(CH2)2-O-(CH2)2-O-(CH2)2-, 39
39, 52%[a]
40, 60%[b]
41, 60%[c]
Ugi MCR using different carboxylic acids to probe the warhead
E
38
18
42
TFE, reflux
X= -(CH2)3-, 12
-(CH2)5-, 14
X= -(CH2)3-, 43
-(CH2)5-, 44
43, 44%
44, 42%
Split Ugi MCR using an aliphatic diamine[d]

[a] Reactions were conducted on a 0.180 mmol scale with respect to **12-16**. Conditions: Reactions were carried out with carboxylic acid **17** or **37** (0.120 mmol, 1 equiv), formaldehyde (37% aqueous solution, 0.360 mmol, 3 equiv), methylamine (40% aqueous solution, 0.240 mmol, 2 equiv), and MeOH (500 μL) at 0 °C for 2 h, then at room temperature for 1 h. [b] Reactions were conducted on a 0.120 mmol scale with respect to **12-16**. Yields over two steps are given. Conditions: 1. Reactions were carried out with carboxylic acid **17** or **38** (0.120 mmol, 1 equiv), paraformaldehyde (0.240 mmol, 2 equiv), tritylamine (0.120 mmol, 1 equiv), and MeOH (500 μL) at 40 °C for 1 h, then overnight at room temperature. 2. Reactions were carried out with TFA (256 μL), and CH_2Cl_2 (256 μL) at 0 °C for 30 min, then at room temperature for 3 h. [c] Reactions were conducted on a 0.120 mmol scale with respect to **12-16**. Reactions were carried out with carboxylic acid **17** or **38** (0.120 mmol, 1 equiv), paraformaldehyde (0.120 mmol, 1 equiv), piperazine (0.120 mmol, 1 equiv), and MeOH (500 μL) heating at reflux for 2 h. [d] Reactions were conducted on a 0.120 mmol scale with respect to **12** and **14**. Reactions were carried out with paraformaldehyde (0.120 mmol, 1 equiv), *N*,*N*-dimethyl-1,3-propanediamine (0.120 mmol, 1 equiv), carboxylic acid **38** (0.120 mmol, 1 equiv), and TFE (500 μL) heating at reflux for 2 h. [1] This reaction was scaled as described in Methods. Color code does not refer to the MCR mechanism, but is intended to represent the synthons and their assemble into the protein degraders.

Different carboxylic acids to probe the warhead.

With the aim of covering a large chemical diversity, we turned our efforts to exploring whether carboxylic acids other than the one based on (+)-JQ1 could be accommodated in the platform and carboxylic acids based on olaparib (poly(ADP-ribose) polymerase PARP inhibitor **37**, Figure 3D) and CX4945 (casein kinase CK2 inhibitor **38**, Figure 3D) scaffolds were selected. In spite of the fact that reactions appeared slower and less clean compared to (+)-JQ1, due to poor solubility of the two carboxylic acids in methanol, three additional PROTACs (**39-41**) were synthesized, in yields comprised between 52 and 60% (Figure 3D). The olaparib-based carboxylic acid was next used to investigate whether a linear aliphatic amine such as *N*,*N*-dimethyl-1,3-propanediamine could be used as an alternative to piperazine in the split Ugi reaction and compounds **43** e **44** were obtained in 44 and 42% of yield, respectively (Figure 3E).

Different isocyanides to probe the anchor.

The use of different isocyanides was explored with the aim of further expanding the chemical space accessible by our platform around the anchor. To investigate whether the Ugi platform could be used to prepare PROTACs directed towards E3 ligases different from CRBN, the isocyanide-based linker bearing the anchor for the VHL protein **45** was prepared by coupling (S,R,S)-AHPC hydrochloride with potassium 2-isocyanoacetate and used in two Ugi reactions using (+)-JQ1 carboxylic acid, affording **46** and **47** in 40 and 42% yield (Figure 4A).

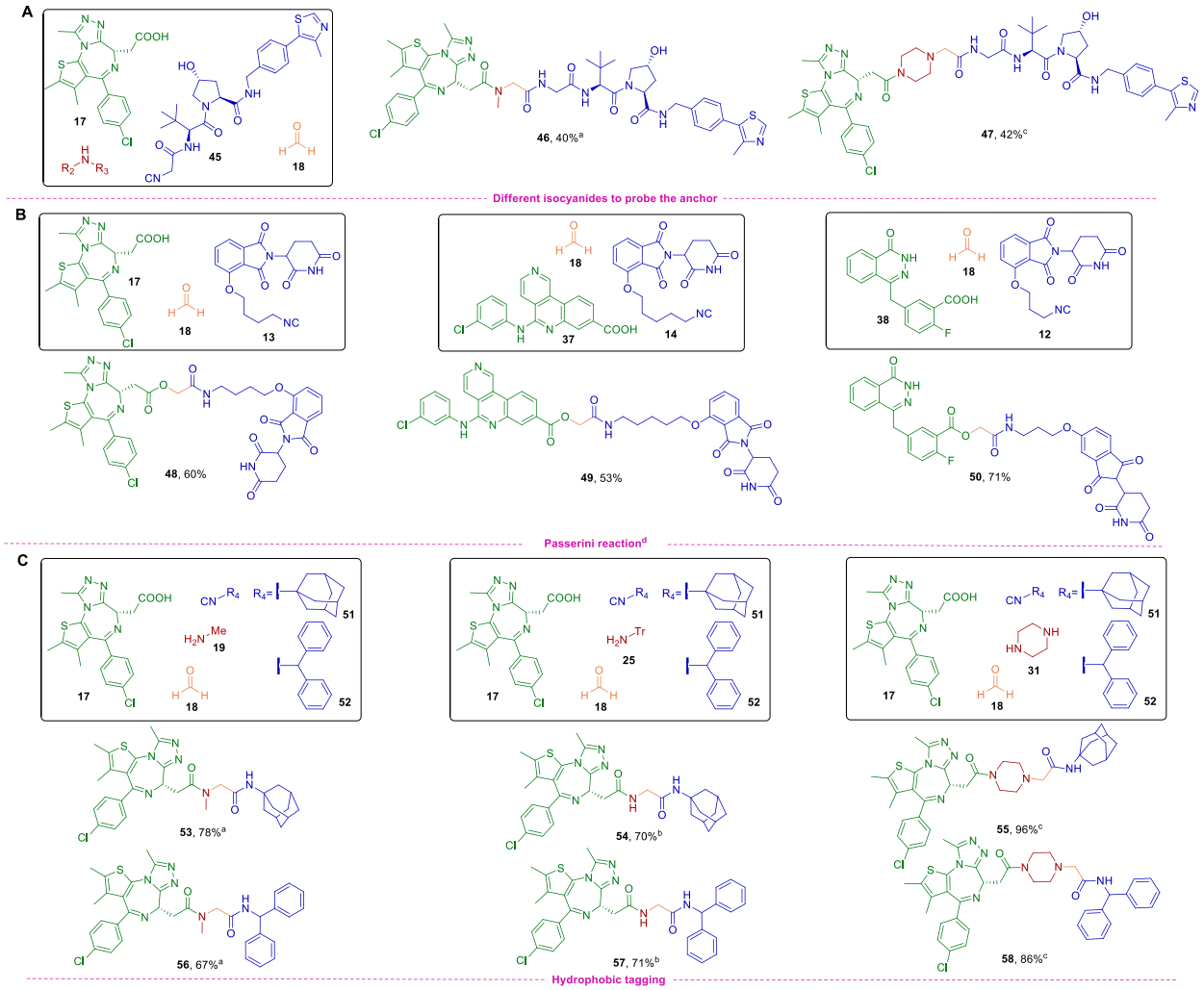
Passerini reaction.

Very recently, Ciulli *et al*. have reported that the bioisosteric replacement of the amide with an ester at the linkage point between the warhead and the linker might enhance the performance of the PROTAC, mainly due to an increase of cell permeability[38]. We therefore investigated the inclusion of the Passerini reaction[39] in the MCR platform and demonstrated that the isocyanide-bearing linkers **12-14** react well in the absence of the amine component and in dichloromethane as solvent to afford **48-50** in 53-71% yield (Figure 4B).

Hydrophobic tagging.

Finally, we leveraged the multicomponent approach to prepare hydrophobic tagging (HyT), a targeted proteolytic system similar to PROTACs. These heterobifunctional degraders display a highly lipophilic moiety, such as an adamantane or diphenylmethane, linked to a ligand for the POI[40,41]. Basically, upon binding to the protein target, the HyT mimics a partially denatured protein folding state, activating the unfolded protein response and inducing degradation of the POI, even if the precise mechanism has not been fully elucidated yet[42]. To this aim, we used commercially available adamantyl (**51**) and diphenylmethane (**52**) isocyanides and prepared in a one-pot fashion six HyTs by the Ugi reaction and its variants (**53-58**, Figure 4C), in 67-96% yield.

Fig. 4 – Changing the anchor, the MCR and the protein degrader subtype.



[a] Reaction was conducted on a 0.180 mmol scale with respect to **45, 51** and **52**. Conditions: Reaction was carried out with formaldehyde (37% aqueous solution, 0.360 mmol, 3 equiv), methylamine (40% aqueous solution, 0.240 mmol, 2 equiv), (+)-JQ1 **17** (0.120 mmol, 1 equiv), and MeOH (500 µL) at 0 °C for 2 h. [b] Reactions were conducted on a 0.120 mmol scale with respect to **51** and **52**. Yields over two steps are given. Conditions: 1. Reactions were carried out with (+)-JQ1 (0.120 mmol, 1 equiv), paraformaldehyde (0.240 mmol, 2 equiv), tritylamine (0.120 mmol, 1 equiv), and MeOH (500 µL) at 40 °C for 1 h, then overnight at room temperature. 2. Reactions were carried out with TFA (256 µL), and CH_2Cl_2 (256 µL) at 0 °C for 30 min, then at room temperature for 3 h. [c] Reaction was conducted on a 0.120 mmol scale with respect to **45, 51** and **52**. Reaction was carried out with paraformaldehyde (0.120 mmol, 1 equiv), piperazine (0.120 mmol, 1 equiv), carboxylic acid (0.120 mmol, 1 equiv), and MeOH (500 µL) heating at reflux for 2 h. [d] Reactions were conducted on a 0.240 mmol scale with respect to **12-14**. Reactions were carried out with formaldehyde (37% aqueous solution, 0.480 mmol, 4 equiv), carboxylic acid **17**, **37** or **38** (0.120 mmol, 1 equiv), and CH_2Cl_2 (1.30 mL) heating at 40 °C for 4 h. Color code does not refer to the MCR mechanism, but is intended to represent the synthons and their assemble into the protein degraders.

Discussion

In this study, we present an unprecedented MCR-based platform that enables the expedient synthetic entry into the chemical space of protein degraders in the scope of green chemistry. When compared to the existing two-components methods, our approach stands out for being:

Reliable, high yielding, protecting group-free and stereoconservative. To give an idea of the simplicity of the approach, in a relatively short period of time, we synthesized a library of more than thirty highly decorated potential protein degraders with an average yield of 65%. The library was prepared starting from a set of CRBN- and VHL-recruiting isocyanides that were easily prepared. Our platform obviates the need to perform protection/deprotection steps typically required in the synthesis of PROTACs, except for the tritylamine when used as a surrogate of ammonia. An additional feature is given by the well-known stereoconservative nature of Ugi and Passerini reactions that is fundamental when chiral warheads and anchors are involved in the assemble of the protein degrader[43,44].

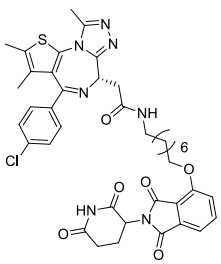
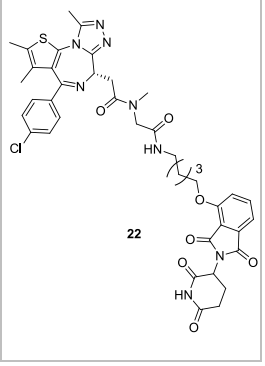
Versatile. By varying the amine component in the MCR, the linkage point between the warhead and the linker is highly diversified, allowing for extensive structure-activity relationship and structure-property relationship studies around not only the linker, its length and composition, but also the attachment point to the warhead, that strongly influences the affinity of the ligand for the POI and is responsible for metabolic instability[15,45]. We have applied this approach to the synthesis of both PROTACs and hydrophobic tagging, depending on the nature of the isocyanide used to perform the MCR, using as model systems different warheads for three POI. Nevertheless, the versatility of MCRs might find other applications, for example in the design of dual or trivalent PROTACs. In the first strategy, two different ligands for two distinct POIs allow for the simultaneous degradation of two proteins in one fashion[46]. In the second, two ligands for the same POI are fused together to engage a more cooperative ternary complex and boost the degradation of the POI[47]. As in both strategies the two ligands are tethered to the E3 ligase-recruiting moiety *via* a branched linker, either the carbonyl

or the primary amine components of the MCR could potentially be further functionalized to act as warhead for the second POI.

Sustainable and cost-effective. The process is characterized by a low environmental footprint. Moreover, except for the (+)-JQ1 based carboxylic acid, all the other substrates required for the preparation of both the linkers and the final degraders are inexpensive and easy handling, in stark contrast with the cost associated with most of the synthons that are currently commercially available[48].

The above advantages become evident when comparing the overall synthetic process required for the synthesis of the PROTAC dBET21[49,50] and its counterpart **22,** that shares the same linker length while displaying one additional amide moiety (Table 1). The patented route to dBET21 requires five steps with an overall yield of 3%, in stark contrast with the MCR platform that allows the synthesis of **22** in four steps with a ten-fold higher yield (30%). The difference is mainly related to the last step that links the warhead to the linker: while the Ugi reaction affords the product in a 65% yield with an atom economy of 73%, the last step for the synthesis of dBET21 is an amide formation reaction mediated by HATU that suffers from a very low yield (10%) and poor atom economy (46%).

Table 1 – Comparative table between a previously reported synthetic approach to PROTACs and this work.

 dBET21	5 (ref. 49,50)	**N° of steps**		4 (this work)	 22
	3%	**Process yield**		30%	
	46%	**Last step**	**Atom economy**	73%	
	10%		**Yield**	65%	
	Prep HPLC+ column chromatography		**Purification**	Column chromatography	

For the time being, we have only scraped the surface of the potential of the platform in the protein degraders field: the described variants have manifold applications and can be easily applied to other proteins for which ligands are available. Making protein degraders easily accessible to chemists at a reasonable cost must be a major objective in the time to come and we envision that our method will help to achieve the goal.

Methods

Materials. Commercially available reagents and solvents were used as purchased, without further purification. When needed, solvents were distilled and stored on molecular sieves. Reactions were monitored by thin layer chromatography (TLC) carried out on 5 cm × 20 cm silica gel plates with a layer thickness of 0.25 mm, using UV light as a visualizing agent. When necessary, TLC plates were visualized with aqueous KMnO4 or aqueous acidic solution of cerium sulfate and ammonium molybdate reagent.

Instrumentations. NMR spectra were recorded on a Bruker Avance Neo 400 MHz (^{1}H and ^{13}C 400 and 101 MHz, respectively) in appropriate deuterated solvents. All protein degraders based on (+)-JQ1 structure were obtained as a mixture of diastereomers which include racemic mixture at the IMiD stereocenter with enantiopure (+)-configuration at the JQ1 stereocenter. IR and HRMS spectra were obtained using FT-IR Bruker Alpha II and (Thermo Fisher Q-Exactive Plus) equipped with an Orbitrap (ion trap) mass analyzer, respectively. Melting points were determined in open glass capillary with a Stuart scientific SMP3 apparatus.

Synthesis of isocyanide 8. Amino alcohol (2.10 g, 20.0 mmol, 1 equiv) **3** and ethyl formate (13.0 mL) were heated under reflux for 6 hours. The volatile was removed in *vacuo*. The corresponding formamide (2.62 g, 20.0 mmol, 1 equiv) was solubilized in dry CH_2Cl_2 (30.0 mL) under nitrogen and, after adding TEA (16.7 mL, 120 mmol, 6 equiv), the reaction mixture was cooled at 0 °C. TsCl (11.4 g, 60.0 mmol, 3 equiv) was added and the reaction was stirred at room temperature for 5 hours. The reaction mixture was quenched with a saturated aqueous Na_2CO_3 solution and stirred under cooling

at 0 °C for 30 minutes. Water was added and the aqueous phase was extracted with CH_2Cl_2 (x3). The organic layer was dried over sodium sulfate and evaporated. The crude product was purified by silica gel column chromatography using PE/EtOAc 9:1, affording compound **8** (3.15 g, 59%) as a yellow oil. See the Supplementary Information for a complete characterization.

Synthesis of isocyanide-based library of CRBN recruiting anchor 14. To a solution of thalidomide derivative **11** (1.65 g, 6.00 mmol, 1 equiv) in dry DMF (25.5 mL), $NaHCO_3$ (756 mg, 9.00 mmol, 1.5 equiv) was added under nitrogen and the reaction mixture was heated at 65 °C. After 15 minutes a solution of isocyanide **8** (1.92 g, 7.20 mmol, 1.2 equiv) in dry DMF (1.50 mL) was added dropwise and the resulting mixture was stirred at 65 °C overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water (x3). The organic layer was dried over sodium sulfate and the volatile was removed in *vacuo*. The crude product was purified by silica gel column chromatography using PE/EtOAc 5:5 as eluent, affording compound **14** (1.88 g, 85%) as a white solid. See the Supplementary Information for a complete characterization.

Scaled-up synthesis of PROTAC 34 by split Ugi reaction. To a solution of piperazine **31** (129 mg, 1.50 mmol, 1 equiv) in MeOH (6.00 mL) paraformaldehyde (72.1 mg, 1.50 mmol, 1 equiv), isocyanide **14** (554 mg, 1.50 mmol, 1 equiv) and carboxylic acid **17** (601 mg, 1.50 mmol, 1 equiv) were added sequentially. The reaction mixture was heated at reflux for 2 hours, then the volatile was removed in *vacuo* and the crude product was purified by silica gel column chromatography using CH_3CN/MeOH 9:1 as eluent, affording compound **34** (1.08 g, 83%) as a white solid. See the Supplementary Information for a complete characterization.

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Author Contributions

M.S. and T.P. conceived and designed the experiments. I.P.B., A.R. and M.S. performed the experiments. I.P.B., A.R. and T.P. analysed the experimental data. T.P. wrote the manuscript with input from all authors. All of the authors have approved the final version of the manuscript.

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